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Lethal and sublethal effects from short-term exposure of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) to diatomaceous earth and spinosad on glass surface

Ehsan PARSAEYAN[1], Moosa SABER[1], Samad VOJOUDI[2,*]

(1. Department of Plant Protection, Faculty of Agriculture, University of Maragheh, Maragheh, 55181-83111, Iran;
2. Young Researchers Club, Marand Branch, Islamic Azad University, Marand, Iran)

Abstract: The cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae), is the main storage pest of cowpea seeds in Iran. Using biorational insecticides instead of conventional insecticides is an appropriate alternative for controlling this pest. In this study, the lethal and sublethal effects of diatomaceous earth and spinosad against *C. maculatus* adults on glass surface were evaluated under laboratory conditions. The results indicated that the LC_{50} values at 24 and 48 h after treatment were 1.47 and 0.2 g/m^2 for diatomaceous earth and 102.9 and 68.8 mg ai/L for spinosad, respectively. Both compounds had high acute toxicity against cowpea weevil. Sublethal effects of LC_{20} concentration of the diatomaceous earth and spinosad against *C. maculatus* adults were studied by detecting biological parameters. LC_{20} concentration of diatomaceous earth and spinosad reduced the fecundity of the pest by 71.5% and 17.2%, the egg hatching rate by 57.5% and 27.8%, and adult longevity by 74.7% and 17.1%, respectively, compared to the control. Pupal period of the insect exposed to LC_{20} concentration of diatomaceous earth and spinosad increased by 4.8% and 2.3%, respectively, compared to the control. The sublethal effect study showed that both diatomaceous earth and spinosad negatively affected life parameters of cowpea weevil. In general, combination of lethal and sublethal effects revealed that diatomaceous earth had high potential in controlling cowpea weevil.

Key words: *Callosobruchus maculatus*; biorational insecticides; life parameters; lethal effects; sublethal effects

1 INTRODUCTION

The cowpea weevil, *Callosobruchus maculatus* (F.) is a serious cosmopolitan and polyphagous pest of stored pulses. The larvae of cowpea weevil feed on stored pulses such as bean, cowpea, lentil, chickpea or other legume grain. The larval stage of the weevil makes tunnel and develops within the beans. The development of a single larva in a grain can lead to weight losses of 8% to 22% (Credland *et al.*, 1986).

Fumigants and residual chemical insecticides such as phosphine, methyl bromide, deltamethrin and malathion are used for controlling *C. maculatus* (Jackai and Adalla, 1997). The use of fumigants and conventional insecticides caused the development of pesticide resistance, hazardous effects to non-target species and beneficial organisms, and environmental and food contamination (Odeyemi *et al.*, 2006; Sadeghi *et al.*, 2006). Therefore, it is necessary to find out appropriate alternative insecticides with safety and low toxicity characteristics to human and environment. Spinosad is a new chemical class of insecticide that is registered by the United States Environmental Protection Agency (EPA) as a "reduced-risk" compound to control a variety of insects. Spinosad is a mixture of two macrolide compounds, *i. e.*, spinosyn A and D, that is synthesized based on the fermentation products of an actinomycete bacterium, *Saccharopolyspora spinosa*. Mode of action of spinosad is by altering the function of nicotinic and GABA-gated ion channels in the postsynaptic cells (Salgado and Sparks, 2010). Previous studies with different formulations of spinosad showed that spinosad could be effective against several stored-product pests (Huang and Subramanyam, 2007; Khashaveh *et al.*, 2009; Vayias *et al.*, 2009; Mollaie *et al.*, 2011).

Diatomaceous earth (DE) obtained from the fossil remains of single celled algae is known as one of the most promising alternatives to traditional residual insecticides (Athanassiou *et al.*, 2008; Vayias and Stephou, 2009). Benefits of DE are the low mammalian toxicity and the nearly negligible resistance development. Diatomaceous earth contains particle pores that absorb lipids from the waxy outer layer of insects' exoskeletons and scratches on the

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[*] Corresponding author, Tel.: +98 421 2273068; Fax: +98 421 2276060; E-mail: svojoudi@yahoo.com

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surface of the cuticle, causing death due to dehydration. Several studies have shown that commercial formulations of diatomaceous earth are effective on stored product pests such as *Sitophillus oryzae* (L.), *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (H.) (Fields and Korunic, 2000, Athanassiou *et al*., 2003; Vayias *et al*., 2006, Kljajié *et al*., 2010).

Effects of insecticides usually are evaluated based on the mortality rate of pests. In addition to direct impact, sublethal effects are those effects that occur in the insecticides exposed survivors. It is indicated that sub-lethal effects of pesticides can affect the physiology and behavior of pests and natural enemies (Johnson and Tabashnik, 1999). Sublethal effects may lead to changes in life table parameters such as longevity, fecundity and population dynamics. Therefore, it was proposed that for evaluating the effectiveness of a pesticide both lethal and sublethal effects should be considered (Stark *et al*., 1998). In this study, the lethal and sublethal effects of spinosad and diatomaceous earth and combination of spinosad and diatomaceous earth were evaluated against cowpea weevil, *C. maculatus* adults under laboratory conditions.

2 MATERIALS AND METHODS

2.1 Insect rearing

Callosobruchus maculatus adults without exposure history to insecticides were obtained from University of Maragheh. Adults of cowpea weevil were reared on the cleared and disinfested cowpea at 25 ± 1℃ and 55% ± 5% relative humidity (RH) in continuous darkness. Young adults (< 24 h) were used in the experiment.

2.2 Insecticides

SilicoSec® is a freshwater formulation of diatomaceous earth from Biofa GmbH and is composed of 92% SiO_2, 3% Al_2O_3, 1% Fe_2O_3 and Na_2O. The median particle size is between 8 – 12 μm. Spinosad (SpinTor® 240SC, Dow AgroSciences, India) was the other insecticide that was used in experiment.

2.3 Bioassay

The toxicity of diatomaceous earth and spinosad was assessed on *C. maculatus*. The range of main concentrations used for bioassay was determined by preliminary dose-setting test. The main experiment was conducted with six different concentrations based on logarithmic interval. Young adults (< 1 day-old) were exposed to spinosad and diatomaceous earth by residue contact method. For cleaving of diatomaceous earth ingredient to petri dishes (9 cm in diameter), petri dishes containing the solution were shaken for 1 min and kept 5 min for deposition of ingredient. After applying the insecticides, about 20 adults of *C. maculatus* were introduced to each petri dish and then transferred to incubator at 25 ± 1℃ and 55% ± 5% RH. Mortality was recorded after 24 and 48 h in all experiments.

2.4 Sublethal effect studies

More than 100 females of *C. maculatus* were exposed to LC_{20} values of each insecticide. After 24 h, the survivors were collected and transferred to incubator with 25 ± 1℃ and 55% ± 5% RH. The experiments were accomplished with 15 replications for tested insecticides and the control (distilled water). Fifteen treated females were transferred individually to a disinfested microtube containing enough cowpea seeds and then an untreated male was added to each microtube. The microtubes and cleared and disinfested cowpeas were substituted daily. The number of eggs laid by females was recorded daily until each female died. Also, other life parameters of cowpea weevil such as egg hatching rate and duration of larval and pupal period were recorded. Experiments were conducted at a temperature of 25 ± 1℃ and 55% ± 5% RH.

2.5 Statistical analysis

Mortality data from the toxicity bioassay were subjected to probit regression analysis. The trials were tested for lack of fit by using PROC GENMOD procedures (SAS Institute, 2002) to compute LC_{20}, LC_{50}, and LC_{90} values on a standard and log scale with associated 95% fiducial limits. Differences in slope between the insecticides were tested using the SAS program (SAS Institute, 2002). Differences in the duration of the larval and pupal stage, fecundity, egg hatching rate and adult longevity were analyzed by ANOVA with mean separation at a 5% level of significance by the Fisher protected least significant difference (LSD) by using SAS software (SAS Institute, 2002).

3 RESULTS

3.1 Acute toxicity bioassay of diatomaceous earth and spinosad against *C. maculates* adults

The results of toxicity bioassay of diatomaceous earth and spinosad against adult stage of the cowpea weevil are presented in Table 1. The results showed that adults of cowpea weevil were susceptible to diatomaceous earth and spinosad. The LC_{50} values reduced with increasing time of exposure in the treatments. The diatomaceous earth and spinosad were more effective after 48 h exposure time compared to 24 h. The effect of exposure time of insecticides tested was significantly different (Table 1).

3.2 Sublethal effects of diatomaceous earth and spinosad on larval and pupal duration and adult longevity of *C. maculatus*

Sublethal effect of LC_{20} concentration of the diatomaceous earth and spinosad on duration of larval and pupal period and adult longevity are presented in Table 2. Diatomaceous earth increased larval and pupal duration significantly compared to spinosad and the control ($F = 8.4$; $df = 2, 38$; $P < 0.001$) (Table 2). Adult longevity of cowpea weevil was significantly different in both treatments in comparison to the control ($F = 93.3$; $df = 2, 44$; $P < 0.0001$). Also, there were significant difference within diatomaceous earth and spinosad. Longevity of adults exposed to diatomaceous earth was lower than spinosad. The longevity of adults exposed to LC_{20} concentration of diatomaceous earth and spinosad was reduced by 74.7% and 17.1% compared to the control, respectively.

Table 1 Toxicity of diatomaceous earth and spinosad against *Callosobruchus maculatus* adults

Insecticide	n	Time of exposure (h)	Slope ± *SE*	χ^2	Lethal concentration (g/m², mg ai/L)		
					LC_{20} (95% FL)	LC_{50} (95% FL)	LC_{90} (95% FL)
Diatomaceous earth	420	24	1.8 ±0.2	59.9	0.51 (0.34 –0.66)	1.47 (1.21 –1.79)	7.30 (5.00 –13.45)
		48	2.5 ±0.3	56.9	0.09 (0.07 –0.11)	0.20 (0.19 –0.23)	0.63 (0.48 –0.99)
Spinosad	420	24	3.3 ±0.4	64.9	57.2 (46.0 –66.3)	102.9 (92.9 –114.8)	251.7 (205.6 –345.4)
		48	2.4 ±0.3	58.9	31.1 (23.0 –37.7)	68.8 (59.7 –80.0)	230.7 (173.3 –366.5)

Lethal concentrations and 95% fiducial limits (FL) were estimated using logistic regression (SAS Institute, 2002).

Table 2 Effects of LC_{20} concentration of diatomaceous earth and spinosad on preimaginal duration and adult longevity of *Callosobruchus maculatus*

Insecticide	Used concentration (g/m², mg ai/L)	Larval and pupal duration (d)	Adult longevity (d)	Mean increase in larval and pupal duration (%)	Mean reduction in adult longevity (%)
Diatomaceous earth	0.51	49.3 ±0.5 a	2.5 ±0.3 c	4.85	74.7
Spinosad	57.2	48.1 ±0.4 b	8.2 ±0.5 b	2.29	17.1
Control	Distilled water	47.0 ±0.2 b	9.9 ±0.4 a	–	–

Means (± *SE*) within a column followed by different letters are significantly different [Fisher's protected Least Significant Difference (LSD), $P < 0.05$]. The same for the Table 3.

Table 3 Effects of LC_{20} concentration of diatomaceous earth and spinosad on fecundity and fertility of *Callosobruchus maculatus*

Insecticide	Used concentration (g/m², mg ai/L)	Fecundity (number of eggs laid per female)	Mean reduction in fecundity rate (%)	Egg hatching rate (%)	Mean reduction in egg hatching rate (%)
Diatomaceous earth	0.51	18.7 ±2.5 c	71.5	25.6 ±3.7 c	57.5
Spinosad	57.2	54.4 ±4.4 b	17.2	43.5 ±4.5 b	27.8
Control	Distilled water	65.7 ±3.0 a	–	60.3 ±3.2 a	–

3.3 Sublethal effects of diatomaceous earth and spinosad on reproduction parameters of *C. maculatus*

Sublethal effect of LC_{20} concentration of the diatomaceous earth and spinosad on fecundity and egg hatching rate of the cowpea weevil are shown in Table 3. Sublethal effect of diatomaceous earth and spinosad caused significant reduction in fecundity and egg hatching rate compared to the control (fecundity: $F = 49.6$; $df = 2, 43$; $P < 0.001$, egg hatching rate: $F = 19.8$; $df = 2, 38$; $P < 0.001$). Percentage reduction of fecundity and egg hatching rate were 71.5% and 57.5% for diatomaceous earth and 17.2% and 27.8% for spinosad, respectively, compared with the control. Also, there was a significant difference within diatomaceous earth and spinosad treatments. Mean daily ovipositon by females in both treatments had a similar pattern and is shown in Fig. 1. Maximum oviposition by females in all treatments was observed in the first few days after emergence.

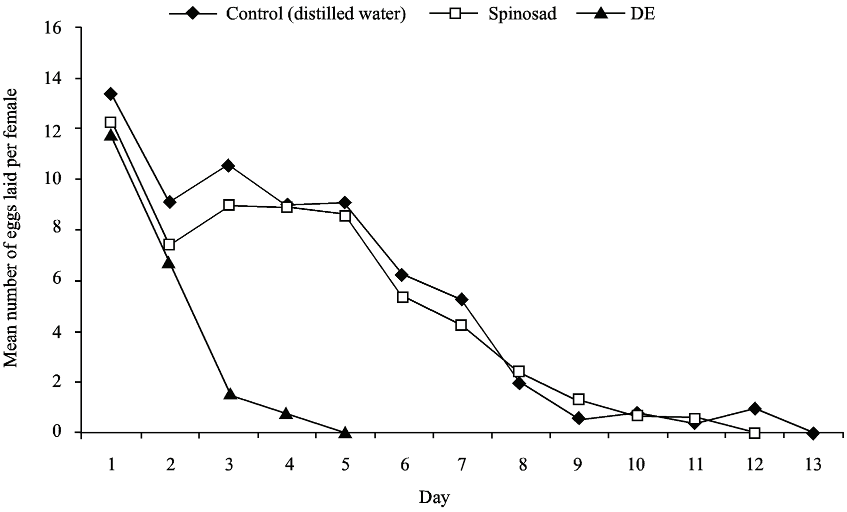

Fig. 1 Mean number of eggs produced daily by a female of *Callosobruchus maculatus* treated with LC_{20} concentration of the diatomaceous earth (DE) and spinosad

4 DISCUSSION

Diatomaceous earth and spinosad exhibited high toxicity to *C. maculatus* as they have been shown to be effective against many other stored pests (Sadat and Asghar, 2006, Vayias *et al.*, 2006; Vardeman *et al.*, 2007; Wakil *et al.*, 2010; Antoine *et al.*, 2010; Kavallieratos *et al.*, 2010). Kamel and Pourmirza (2006) stated that liquid formulation of spinosad had good efficacy against *C. maculatus* adults and it may be an alternative biopesticide for controlling this pest. In their study, spinosad caused 75% - 100% mortality at the application rates of 96, 72, 44.4 and 36 mg ai/L. Wakil *et al.* (2010) also reported that treatment of *Rhyzopertha dominica*, *T. castaneum* and *S. oryzae* with 0.5 g/m^2 of diatomaceous earth caused about 83% mortality after 24 h. In our study, the results obtained from acute toxicity assays showed that diatomaceous earth and spinosad were infective on adults of cowpea weevil. The toxicity of tested insecticides increased with increasing exposure time in the treatments. Spinosad and diatomaceous earth did not cause an immediate knock-down effect and were comparably slow acting.

Environmental condition such as humidity may affect the efficacy of diatomaceous earth. Collins and Cook (2006) evaluated the effects of diatomaceous earth on glass surface against *Sitophilus granarius* and *Oryzaephilus surinamensis* at 15℃ and 80% RH. They reported that Silico-sec dry dust at the application rate of 5, 10 and 20 g/m^2 caused 99% - 100% mortality after 7 d. In the present study, diatomaceous earth at the application rate of 0.5 - 1 g/m^2 caused about 90% mortality on cowpea weevil at 25℃ and 55% RH after 48 h. Differences in the efficacy of diatomaceous earth in these two studies would be due to differences in the species of insects and environmental condition especially humidity and temperature. Also Cook (2003) recorded 100% mortality of *T. castaneum* and *S. granaries* when exposed to 1 - 10 g/m^2 diatomaceous earth for 48 h, followed by a 7 day recovery period in the condition of 30℃ and 50% RH. Therefore, an increase in the humidity content and reduction of temperature of stores will considerably reduce the efficacy of diatomaceous earth (Korunic, 1997). Many studies have been conducted about long term toxicity of spinosad and diatomaceous earth to stored pests, but few studies have been published about sublethal effect of these insecticides on stored pests. In this study, we found that sublethal concentration of diatomaceous earth and spinosad reduced the fecundity, adult longevity and egg hatching rate of the pest. They also increased larval and pupal duration of cowpea weevil (Tables 2, 3). Shayesteh and Ziaee (2007) reported that reproduction potential of adult *T. castaneum* was significantly suppressed in treatments containing silico-sec. These negative effects of diatomaceous earth on life stages of cowpea weevil could be due to absorbing lipids on the surface of the cuticle that caused dehydration of body. Exposure to sublethal concentration of spinosad also caused reduction in fecundity, egg hatching rate and adult longevity and increase in larval and pupal duration of cowpea weevil compared to the control. Several studies indicated that spinosad reduced the progeny production of several stored pests such as *R. dominica*, *S. oryzae*, and *S. granaries* (Kavallieratos *et al.*, 2010; Athanassiou, *et al.*, 2011). Antoine *et al.* (2010) reported that spinosad caused high mortality on adult *C. maculatus* and decreased the number of eggs laid by females. In that study, spinosad also significantly reduced egg hatching compared with the untreated control. Thus, spinosad is an appropriate alternative biopesticide to the synthetic pesticides due to its high acute toxicity to stored pests especially cowpea weevil, and negative effects on life parameters of the pest as well as its harmlessness to mammals and environment (Thompson *et al.*, 2000).

In addition, we demonstrated that diatomaceous earth and spinosad had high acute toxicity to cowpea weevils. Both compounds negatively affected the life parameters of cowpea weevil such as fecundity, egg hatching rate and adult longevity. These effects are very important in pest management, because delayed effects of diatomaceous earth and spinosad will cause the reduction in offspring number and consequently reduction in insect population in future slip. Therefore, we concluded that diatomaceous earth and spinosad had high potential in controlling cowpea weevil.

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在玻璃表面上短时接触硅藻土和多杀菌素对四纹豆象的致死效应和亚致死效应

Ehsan PARSAEYAN[1], Moosa SABER[1], Samad VOJOUDI[2,*]

(1. Department of Plant Protection, Faculty of Agriculture, University of Maragheh, Maragheh, 55181-83111, Iran;
2. Young Researchers Club, Marand Branch, Islamic Azad University, Marand, Iran)

摘要: 四纹豆象 *Callosobruchus maculatus* (F.)是伊朗豇豆种子上的主要贮藏害虫。控制这一害虫时，用生物杀虫剂比用常规杀虫剂更为合适。本研究评价了室内条件下在玻璃表面上硅藻土和多杀菌素对四纹豆象成虫的致死效应和亚致死效应。结果表明：硅藻土处理24 h和48 h后，对四纹豆象成虫的LC_{50}值分别为1.47和0.2 g/m^2；多杀菌素处理24 h和48 h后，对四纹豆象成虫的LC_{50}值分别为102.9和68.8 mg ai/L，说明两种化合物都对四纹豆象成虫具有较高的急性毒性。通过检测生物学参数，研究了LC_{20}浓度的硅藻土和多杀菌素对四纹豆象的亚致死效应。LC_{20}浓度的硅藻土和多杀菌素使四纹豆象成虫的繁殖力分别比对照降低了71.5%和17.2%，卵孵化率降低了57.5%和27.8%，成虫寿命缩短了74.7%和17.1%。接触LC_{20}浓度的硅藻土和多杀菌素使这一害虫的蛹期分别比对照延长了4.8%和2.3%。亚致死效应研究表明，硅藻土和多杀菌素对四纹豆象的生命参数均产生了负面影响。总之，致死效应和亚致死效应综合显示，硅藻土在防治四纹豆象上具有较大的潜力。

关键词: 四纹豆象；生物杀虫剂；生命参数；致死效应；亚致死效应

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